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Electroporation is a dramatic and apparently universal phenomenon which occurs in all bilayer-containing membranes. For this reason electroporation has implications for basic understanding of cell membranes, and is also likely to lead to a number of new applications. A quantitative understanding of how electroporation occurs has been lacking. We have made significant progress towards providing descriptions of mechanisms which can quantitatively account for most of the complex electrical behavior of planar bilayer membranes without proteins, and also molecular transport due to electroporation drift. This has set the stage for development of models which describe both electrical behavior and molecular transport of both plane membranes, and of cell membranes. Although originally unanticipated, we were also able to quantitatively estimate the thermal noise limit for possible weak electric field effects in living cells, and showed that the "kT limit" could be small. JJS

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INTRODUCTION

Our objective has been the development of a quantitative theory of the mechanism of electroporation. Overall we have sought to develop models which allow quantitative descriptions of measurable quantities. This has included the more specific objective of first quantitatively describing key features of electrical behavior due to electroporation, and then molecular transport under the same conditions. Although not originally anticipated, during the course of our work we realized that the question of "signal-to-noise ratio" could be applied to the interaction of electrical fields with cells. This has allowed us to expand our work to also include an estimate of the "thermal noise limit" for the response of living cells to weak electrical fields.

Electroporation (i) is presently believed to be a universal cell membrane phenomenon, involving both the lipid bilayer and membrane macromolecules, and is therefore fundamental to membrane understanding, (ii) provides a general method for introducing molecules into cells, or releasing molecules from cells, with potentially major applications in science and technology, and yet (iii) the basic mechanism is incompletely understood.[1-3] For example, no previous theoretical model actually described electrical behavior (e.g. transmembrane potential as a function of time), membrane recovery (e.g. membrane conductance vs. time after reversible electrical breakdown), or the net molecular transport (e.g. number of molecules transported across the membrane) associated with a short pulse which causes electroporation. We have succeeded in obtaining such descriptions, and they agree reasonably with the experiments which allow a quantitative comparison.

In the case of weak electric field thresholds, there has been much controversy regarding whether or not reported effects are real. One specific objection has been that thermal fluctuations would overwhelm the effects of weak fields. We have assumed that a true response by a cell corresponds to a physical detection event, so that the use of a signal-to-noise ratio could be used ("signal" = applied electric field; "noise" = confounding effects cast into an equivalent electric field). By considering Johnson noise in the cell membrane, we were able to estimate the thermal noise limit for possible weak field responses, and found that this limit can correspond to small external electric fields. This does not prove that weak field effects occur, only that one previously stated objection does not apply.

QUANTITATIVE THEORETICAL MODELS FOR ELECTROPORATION

Our specific goals for electroporation theoretical models have been:

(1) Extension of an initial theory of reversible electrical breakdown to one with a more solid foundation. Specifically, we have succeeded in eliminating the need for an approximate "switch on" criteria of pores, and for the assumption of the membrane containing so many pores that the

membrane was "geometrically saturated" with pores. The first results are presented in a recent paper.

(2) Quantitative description of the transmembrane potential, U(t), during both reversible electrical breakdown (REB) and irreversible rupture, has been achieved. In this sense, a unified theoretical description of both REB and rupture is provided by one model. The model can actually describe quantitatively the <u>four</u> observed fates of a artificial planar bilayer membrane under "charge injection" (very short pulse) conditions. For a short square pulse these four fates are (in order of progressively smaller pulse magnitude):

- Reversible electrical breakdown (REB) leading to complete membrane discharge
- Incomplete REB (discharge halts at $U > 0$)
- Rupture (mechanical) with its characteristic slow, sigmoidal electrical discharge
- Membrane charging without dramatic behavior at small U

This theoretical model has yielded predictions of U(t) which compare favorably with "charge-injection" experiments (see plots below). [4] , and represents achievement of a basic goal of our study.

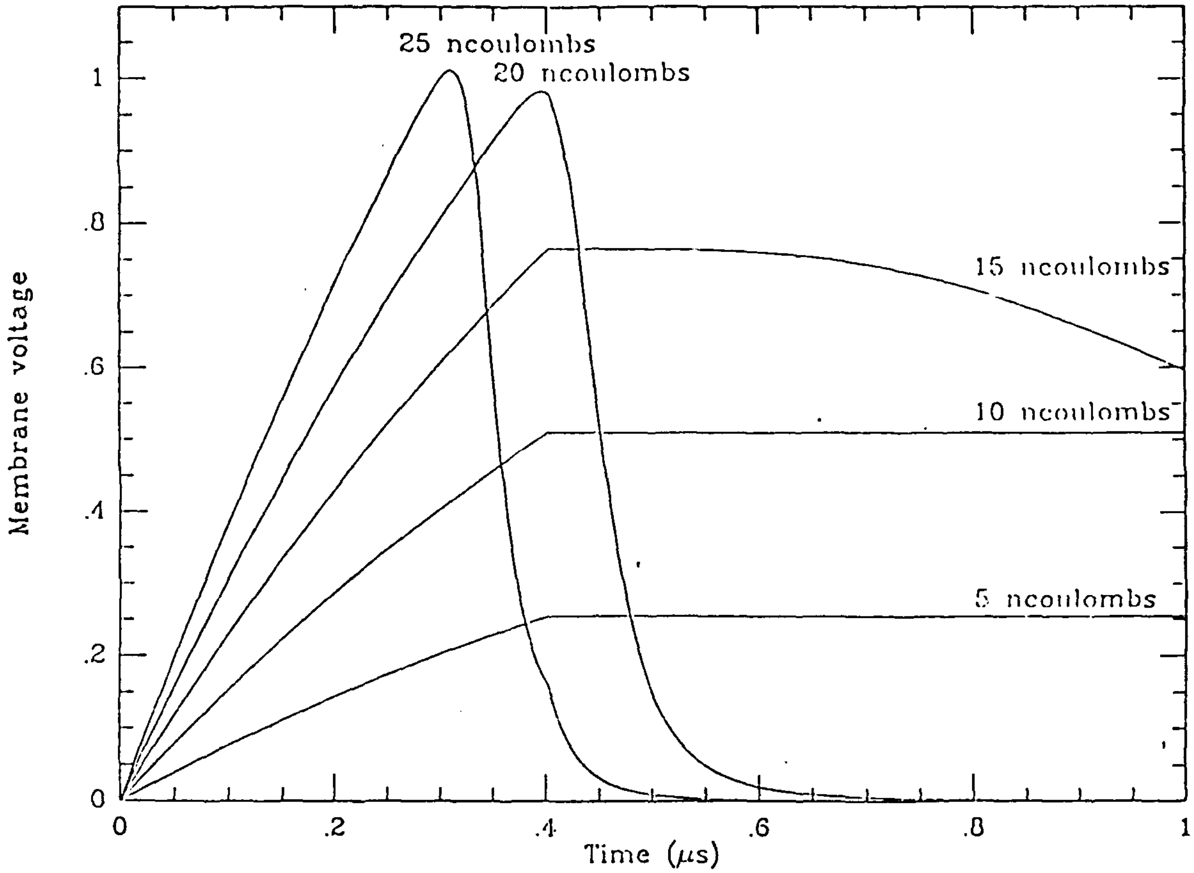

Fig. 1: Short time scale (0 to 1µseconds) electrical behavior predicted by the theoretical model. The 5 and 10 ncoul curves represent simple charging of the membrane (on this time scale). The 15, 20 and 25 ncoul curves various degrees of "breakdown" (actually a high conductance state), with larger pulses resulting in progressively higher conductance, and therefore faster discharge after the pulse.

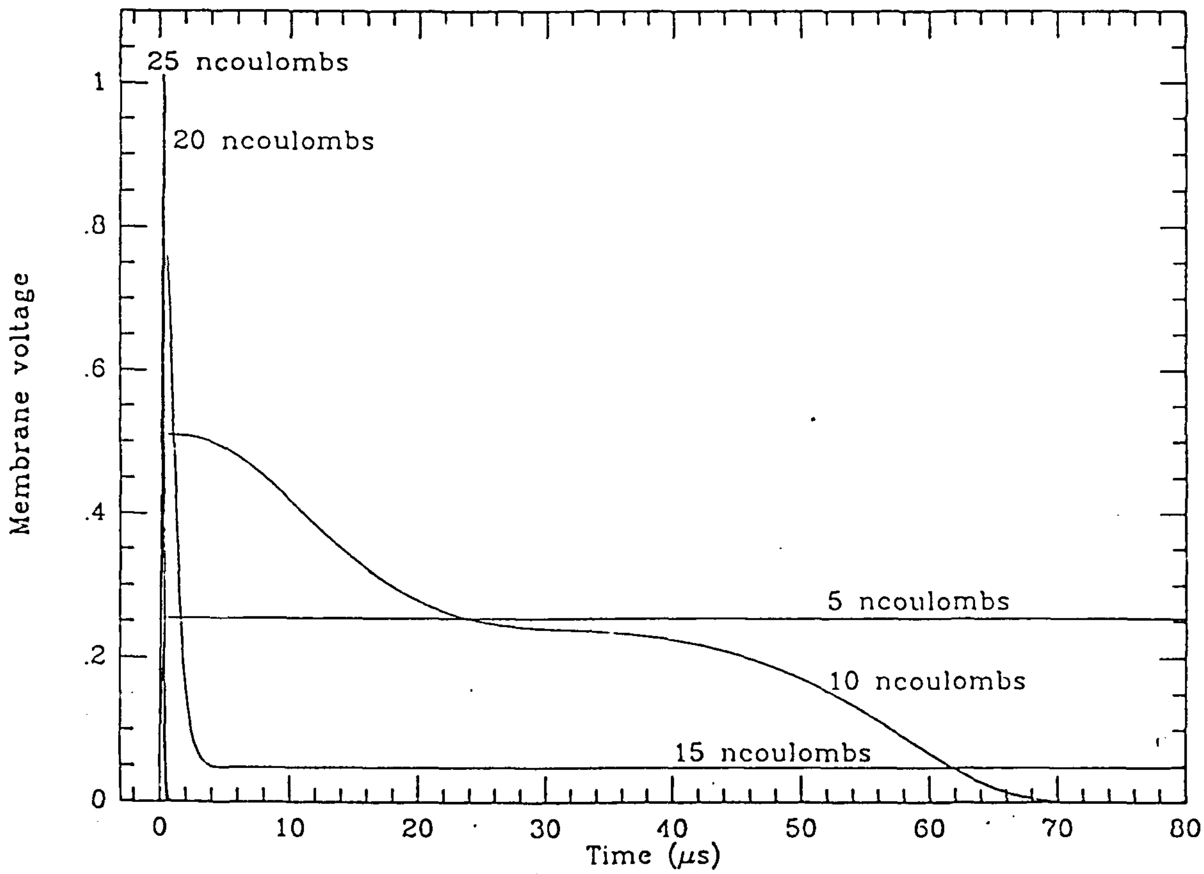

Fig. 2: Longer time scale (0 to 80μseconds) electrical behavior predicted by the same model. The 5 ncoul curve corresponds to a charged membrane, while the 10 ncoul curve is (delayed) rupture. The 15 ncoul curve is incomplete reversible electrical breakdown in which the discharge does not reach zero (the final transmembrane potential ≈ 50mV). The 20 and 25 ncoul curves are reversible electrical breakdown (REB), in which the membrane discharges completely because of ionic conduction through a large, dynamic pore population, and then recovers to its original state.

(3) Molecular transport has also been described quantitatively by an extension of the model. In this case the model predicts both electrical behavior and the amount of transmembrane transport of molecules. In these first molecular transport calculations we have only included the contribution of electrophoretic drift through a dynamic population of pores of many sizes.[5-7] Here the involvement of membrane proteins, particularly channel forming proteins, is believed to be important. Contrary to our initial expectations, diffusion is unlikely to be the primary mechanism for molecular transport over short times. However, for long times the involvement of persistent metastable pores will probably predominantly involve hindered diffusion. Electroosmosis which may also operate during the time the transmembrane potential is non-zero, and should be incorporated in future versions of the model. Overall, our goal has been to predict the number of molecules which move across a cell membrane, and also (because of the fundamentally statistical nature of the theory, and the statistical orientation of non-spherical cells) the distribution of transport within a cell population.[8] Initial success has been achieved with former, but not yet the latter. In order to model transport, we have used a version of the planar membrane model with a very small surface energy, as this is equivalent to recognizing that a cell membrane cannot experience prompt

rupture through formation of a supracritical pore.[9] Recently, a still better approximation for a cell has been identified which involves two small planar membranes in series.[10]

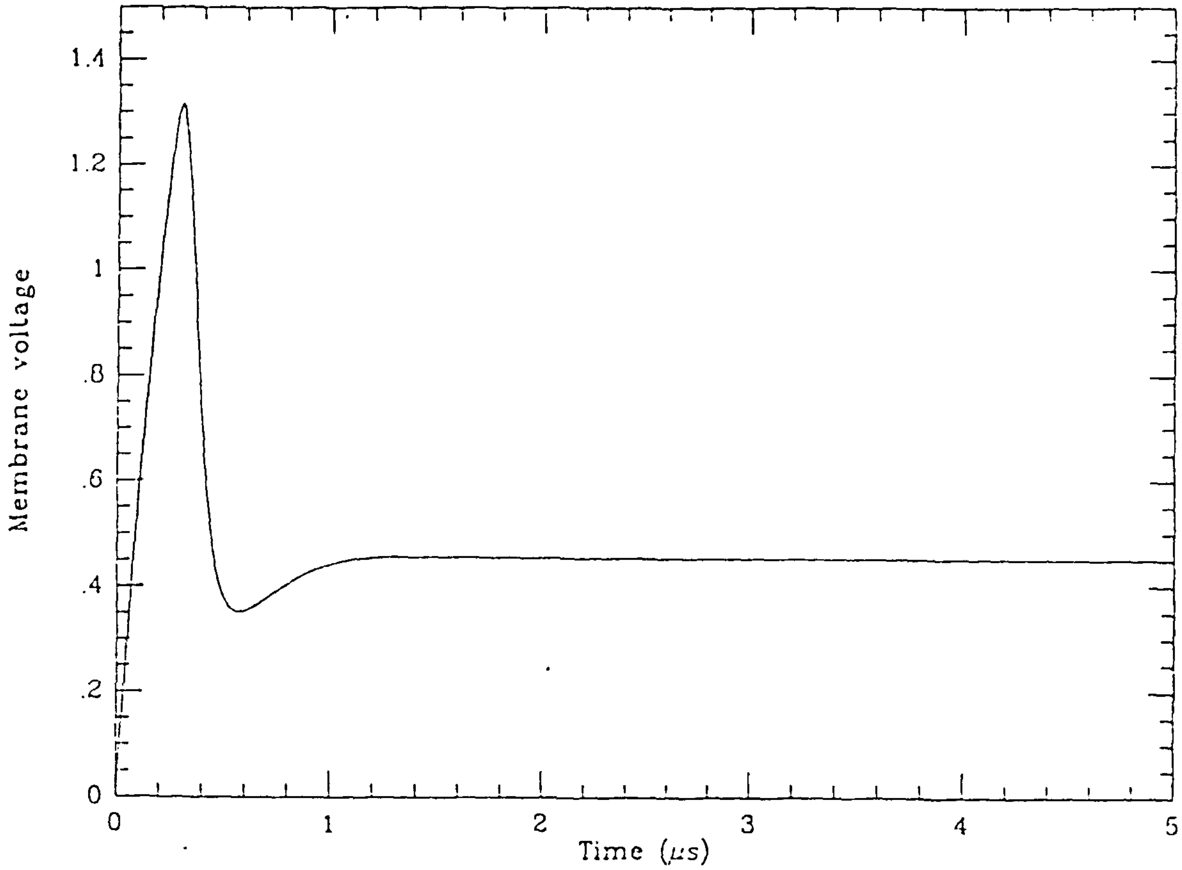

Fig. 3: The first 5 μseconds of the transmembrane potential versus time in response to a voltage square wave of amplitude 2.5 volts and duration 100 μseconds. The transmembrane potential increases rapidly to ≈ 1.2 volt, and then decreases as reversible electrical breakdown (REB) occurs. The decreasing potential stops and then briefly increases, as a steady state voltage divider effect involving the external source resistance and the membrane resistance becomes dominant. While the pulse is still on U(t) approaches as asymptotic value of ≈ 0.46 volt. This simulation models a portion of a cell membrane, for which prompt rupture via a supracritical pore cannot occur (modelled here by making the membrane surface energy very small, i.e. $\Gamma = 10^{-5}$ joule m^{-2}).

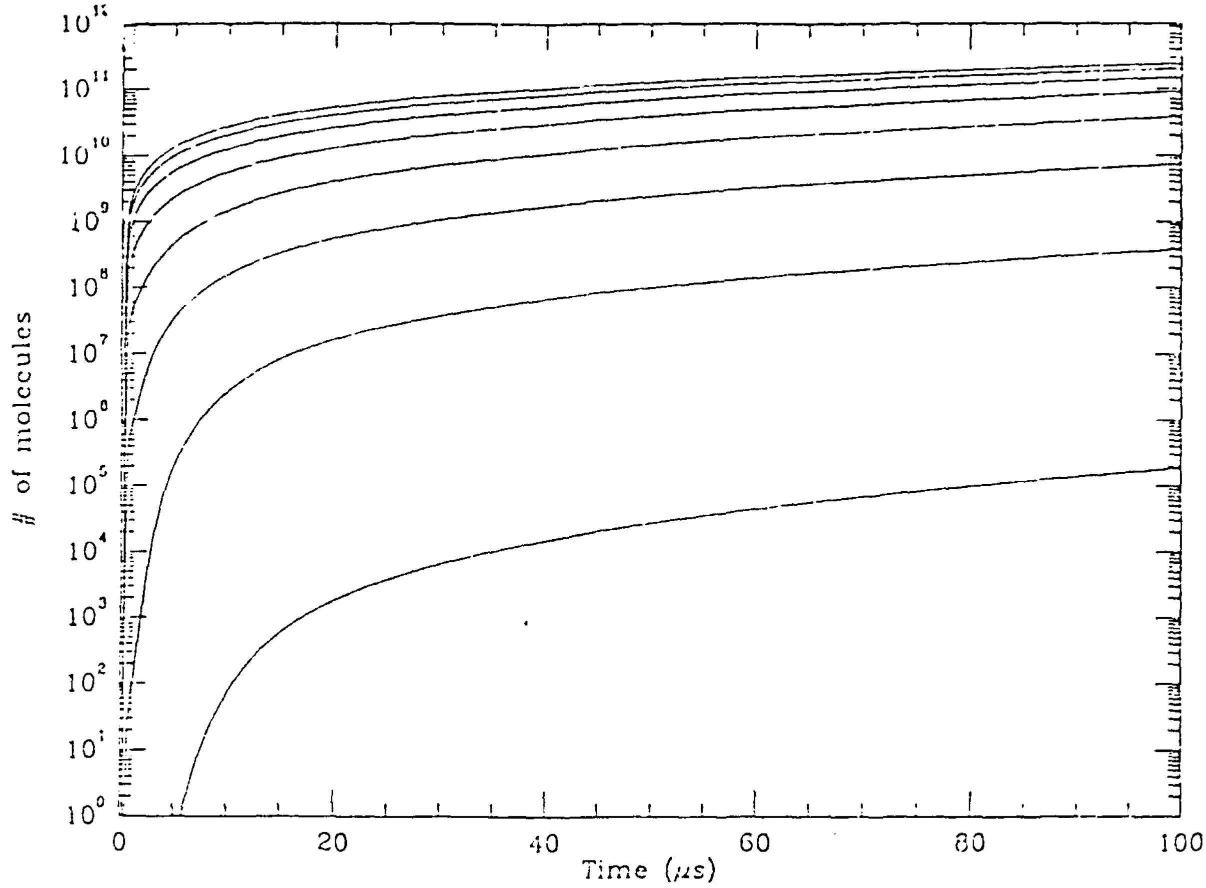

Fig. 4: Integrated flux versus time for molecules of radius 1.8 nm and different charges for the full 100 μseconds. The curves are for z = 1, 3, 5, 7, 10, 20, 30, and 40, where z is the number of proton charges on the molecule. For $z < 10$, the effect of charge is quite weak, but transport of molecules with $z > 10$ is much smaller. This computation shows the importance of charge in excluding molecules with large net charge, for which the Born energy exclusion effect is large, and becomes more important that the electrophoretic driving force. Future simulations of this type should lead to predictions which can be compared to experiments with cells.

(4) Metastable pores have been considered for inclusion in an extended version of this theoretical model. For example, pores associated with a "foot-in-the-door" mechanism has been qualitatively considered, and can now be approached quantitatively in future work. As envisioned, this extension is based on a molecule/pore interaction wherein the presence of a macromolecule partially inserted within a pore prevents the pore from shrinking and then vanishing. The macromolecule within the pore can be a cytoplasmic macromolecule or can be associated with a cytoskeleton, in which case the macromolecule holds the pore open while other, smaller molecules are transported.

(5) Pore-membrane macromolecule interactions have also been considered as the basis of a possible extension of the theoretical model. Membrane channel proteins are prime candidates for such interactions, and may provide nucleation sites for the long lifetime pores which are believed to occur. Such metastable pores may have significantly longer lifetimes than "ordinary" transient aqueous pores, and may exhibit a strong temperature dependence of the lifetime. This candidate

mechanism may also be relevant to describing molecular transport, through providing longer pore lifetimes.

Throughout we have sought quantitative estimates which can be compared to the results of experiments by ourselves and others.

WEAK FIELD THRESHOLD RESPONSE OF LIVING CELLS

Although not originally anticipated, an outgrowth of our work has been consideration of some of the basic signal-to-noise ratio issues that should govern the response of cells to weak electrical fields. [11,12] In this part of the study, we have considered:

(6) The fluctuations in transmembrane potential due to Johnson noise, as described by Nyquist's theoretical model.[13] These "kT" fluctuations in potential can be small, because for low frequencies the entire membrane is coherently involved. One simple result is that larger cells should have smaller thermal noise.

(9) The minimum strength external field, which corresponds to a signal-to-noise ratio equal to one, was calculated. This estimate was obtained by directly comparing the rms thermal noise to the maximum change in transmembrane potential caused by an external electric field. The broad band case was considered for both spherical and elongated cells, with moderately small threshold fields predicted.

(8) Smaller noise, and therefore smaller thresholds, were expected if the relevant frequency bandwidth was smaller than the broad band case. The possibility of an averaging process was also explored, with the specific mechanism of electroconformation of membrane enzymes considered as an example. If an averaging process is involved, significantly smaller thresholds are expected.

(9) Free (soluble) molecules were estimated to respond at far larger electrical fields than membrane molecules. This estimate was obtained by comparing the end-to-end thermal noise for a linear molecule to the maximum potential difference that an external electric field could cause along the length of the molecule. This estimate strengthens the view that the likely site of weak electric field interactions is the cell membrane, where interfacial polarization results in a significant amplification of the external electric field.

SUMMARY

We have successfully completed an extension of a transient aqueous pore theory of electroporation. This improved model yields descriptions of four key aspects of complex electrical behavior in artificial planar bilayer membranes, allows prediction of the electrophoretic contribution to molecular transport across planar bilayer or cell membranes, and sets the stage for considering other types of transport (e.g. hindered diffusion, electro-osmosis) through a changing population of pores. In addition, weak electric field thresholds for the response of non-specialized cells has been quantitatively estimated using a signal-to-noise ratio criterion. Throughout we have emphasized theoretical modelling which can lead to the quantitative prediction of experimental results, thereby allowing direct comparisons between experiments and theories.

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